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Abstract: AIMS: The aim of this present study was to investigate the changes of peripheral sensory nerve excitability produced by propofol. MAIN METHODS: In a recently described in vitro model of rodent saphenous nerve we used the technique of threshold tracking (QTRAC®) to measure changes of axonal nerve excitability of A -fibres caused by propofol. Concentrations of 10 Mol, 100 Mol and 1000 Mol were tested. Latency, peak response, strength-duration time constant (SD) and recovery cycle of the sensory neuronal action potential (SNAP) were recorded. KEY FINDINGS: Our results have shown that propofol decreases nerve excitability of rat primary sensory afferents in vitro. Latency increased with increasing concentrations (0 Mol: 0.96±0.07ms; 1000 Mol 1.10±0.06ms, P<0.01). Also, propofol prolonged the relative refractory period (0 Mol: 1.79±1.13ms; 100 Mol: 2.53±1.38ms, P<0.01), and reduced superexcitability (0 Mol: -14.0±4.0%; 100 Mol: -9.5±5.5%) and subexcitability (0 Mol: 7.5±1.2%; 1000 Mol: 3.6±1.2) significantly during the recovery cycle (P<0.01). SIGNIFICANCE: Our results have shown that propofol decreases nerve excitability of primary sensory afferents. The technique of threshold tracking revealed that axonal voltage-gated ion channels are significantly affected by propofol and therefore might be at least partially responsible for earlier described analgesic effects.

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Propofol Decreases The Axonal Excitability In Rat Primary Sensory Afferents

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Abstract

Aims:

The aim of this present study was to investigate the changes of peripheral sensory nerve excitability produced by propofol.

Main methods: In a recently described in vitro model of rodent saphenous nerve we used the technique of threshold tracking (QTRAC®) to measure changes of axonal nerve excitability of Aβ-fibres caused by propofol. Concentrations of 10 µMol, 100 µMol and 1000 µMol were tested. Latency, peak response, strength-duration time constant (τSD) and recovery cycle of the sensory neuronal action potential (SNAP) were recorded.

Key findings: Our results have shown that propofol decreases nerve excitability of rat primary sensory afferents in vitro. Latency increased with increasing concentrations (0 µMol: 0.96 ± 0.07 ms; 1000 µMol 1.10 ± 0.06 ms, $P<0.01$). Also, propofol prolonged the relative refractory period (0 µMol: 1.79 ± 1.13 ms; 100 µMol: 2.53 ± 1.38 ms, $P<0.01$), and reduced superexcitability (0 µMol: -14.0 ± 4.0%; 100 µMol: - 9.5 ± 5.5 %) and subexcitability (0 µMol: 7.5 ± 1.2 %; 1000 µMol: 3.6 ± 1.2) significantly during the recovery cycle ($P<0.01$).

Significance: Our results have shown that propofol decreases nerve excitability of primary sensory afferents. The technique of threshold tracking revealed that axonal voltage-gated ion channels are significantly affected by propofol and therefore might be at least partially responsible for earlier described analgesic effects.

Key words: propofol; nerve excitability; sensory afferents, threshold tracking

Introduction

A recent report described short-lasting analgesic properties of propofol during its administration in a human pain model (Bandschapp et al., 2010). The mechanisms behind this effect, however, remain obscure. In theory, modulation of the nociceptive signalling by propofol can take place in both, the peripheral and central nervous system. As an effective general anesthetic propofol is acting on the transmission of action potentials on neuronal synapses in the central nervous system (CNS). The primary target of propofol is the $GABA_A$ receptor which is abundantly expressed in the CNS. But propofol also activates transient receptor potential (TRP) receptors TRPA1 and TRPV1 on nociceptors and may lead to a pain sensation at high concentrations (Fischer et al., 2010; Picard and Tramer, 2000). Previous studies have shown that propofol also acts on voltage-gated ion channels involved in the excitability of neuronal and muscular cell membranes even at systemic concentrations reached during anesthesia (Haeseler et al., 2001). Possible mechanisms include shortening of the opening of voltage-gated sodium channels and a prolonging of their steady-state inactivation in the CNS (Frenkel and Urban, 1991). Additionally, propofol shifts the inactivation of sodium channels towards more hyperpolarizing potentials, resulting in suppression of excitability (Rehberg and Duch, 1999). Although these results are not fully conclusive since they describe effects on different subtypes of voltage-gated sodium channels either in skeletal muscle ($Na_V1.4$), or in the central nervous system ($Na_V1.1$, $Na_V1.2$, $Na_V1.3$), they also suggest a possible role of the subtype $Na_V1.6$ in myelinated nerve fibers (Kakinohana et al., 2002; Dueck et al., 2003). The necessary concentrations to elicit these effects in the afore mentioned studies were all below 100 µM and therefore close to the therapeutic range in a setting of general anesthesia (Kazama et al., 1997). A recent clinical investigation showed that excitability of peripheral sensory Aβ-afferents which mainly involves subtype $Na_V1.6$ is altered during the induction of anesthesia with propofol (Maurer et al., 2010). In this study, a direct action of propofol on peripheral excitability was proposed, however an interfering temperature effect could not be excluded.

The purpose of this present study was to investigate a direct effect of propofol on axonal nerve excitability in a setting where a possible temperature effect can be controlled. We used the technique of threshold tracking in an in- vitro model of rodent saphenous nerve which simulates accurately the changes which occur in human models in a qualitative manner (Maurer et al.,

2007). This technique provides information about axonal function of peripheral nerves in contrast to routine nerve conduction studies which is measuring amplitude and velocity only.

Materials and Methods

Ethics: All the animal work was done in accordance to the *Swiss Animal Protection Act 2008* and after approval of the *Health Department of the Canton of Zurich, Switzerland, reg. nr. 217/2007.* A total of 13 nerves of 13 adult female Wistar rats were used in the investigation. The animals were killed by 100 % CO_2. The saphenous nerve and its innervated skin were excised from hind limbs and kept corium-side up in an organ bath (Fig. 1). The preparation was superfused by synthetic interstitial fluid (SIF, in mmol/l): 124 NaCl, 3.5 KCl, 0.7 $Mg(H_2O)7$, 1.65 NaH_2PO4, 2 $CaCl_2$, 9.6 sodium gluconate, 5.55 glucose and 2.9 sucrose. The pH of the SIF was maintained at 7.40 by continually gassing with a mix of 95% oxygen and 5% carbon dioxide (carbogen). The temperature of the bath was maintained at 32°C which corresponds to a physiological temperature of a cutaneous nerve. The nerve's proximal end was desheathed 1mm and placed in a separate chamber isolated by silicone oil. Recordings were obtained with gold wire electrodes where the cathode served as an active electrode and the anode as reference electrode positioned nearby in the SIF. Generated action potentials were recorded with an isolated bio-amplifier (ISO-80, World Precision Instruments, Hertfordshire, UK). Recordings were started only after the nerve has been in the organ bath for an equilibrating period of 2 hours.

A 1 % propofol stock solution (10mg/ml; Disoprivan®; AstraZeneca AG, 6301 Zug, Switzerland) was dissolved in SIF to make up the working solutions of different concentrations (e.g. 0.089 mg/l = 500 µM; MW = 178.2). To separate the effects of the active component propofol and the vehicle intralipid (Sojae oleum 100 mg/ml, 1.2% purified egg phospholipid, 2.25% of glycerol) we tested the effect of vehicle alone (Intralipid® 10%; Fresenius Kabi, 6371 Stans, Switzerland). In these preliminary experiments (n = 5) intralipid (100 mg/ml) was dissolved in SIF analogously in a concentration equivalent to the tested propofol. The nerve was cut, desheathed over 3 mm and stimulated with a silver wire within a suction electrode. During continuous recording of control threshold and superexcitability (s. paragraph below, 'threshold tracking'), intralipid alone was first washed in for 15 minutes. Subsequently, after a washout of 10 minutes 500 µM propofol was washed in for 15 minutes, followed by a washout period.

We applied propofol using concentrations between 10µM and 1 mMol. The recording setting was changed in the following manner: We left the nerve attached to the skin and stimulated using an Ag/AgCl electrode with an uninsulated tip diameter of 2 mm (EPO5, World Precision Instruments, Hertfordshire, UK) with the cathode positioned within the ring and the anode put 2-3 cm outside the ring in the organ bath (Fig. 1). In contrast to the stimulation with suction electrodes, this arrangement allows better to perform excitability measurements with stimulation protocols which include polarizing conditioning stimuli. Different concentrations of propofol (0 µMol 10 µMol, 100 µMol and 1000 µMol) were applied within the ring using a circulating system which was independent of the rest of the organ bath. Analogously to the circulation system of the organ bath, the SIF containing the active component was continually gassed with carbogen and also maintained at 32 ˚C. Cumulative concentration-response studies were conducted on each nerve preparation. Each concentration was washed in for at least 40 minutes in order to reach a steady state. During the actual recording of the excitability parameters the content of the ring was replaced by silicone oil (32 ˚C) containing the same concentration of propofol as the SIF washed in immediately before.

For both sets of experiments, the wave form of the stimulus was generated by the software QTRAC© (Institute of Neurology, University College London, UK) and converted to current using an isolated linear constant current stimulator (Linear Stimulus Isolator A395, World Precision Instruments, Hertfordshire, UK). Generated action potentials were recorded with an isolated bio-amplifier (ISO-80, World Precision Instruments, Hertfordshire, UK) from the proximal nerve's ending using the following settings: low pass filter: 2000 Hz; high pass filter: 1 Hz; gain 1000x. Data were then digitized by a data acquisition unit (CED micro1401 MK II, Cambridge Electronic Design Ltd, Cambridge CB4 0FE, UK) using a sampling rate of 10 kHz. All measurements with propofol were carried out in concentrations of ascending order. Upon completion, the recording site within the ring was washed out with SIF alone for 90 minutes and followed by a final recording.

Threshold tracking

We used a computer-assisted threshold tracking program (QTRAC© Institute of Neurology, Queen Square, London, UK) to investigate the excitability parameters and ion conductances in myelinated sensory axons in peripheral nerves (Maurer et al., 2010; Bostock et al., 1998; Z'graggen and Bostock, 2008). The recording protocols allow to measure specific

electrophysiological parameters. Detail of the protocols, how the measurements were plotted and interpreted are extensively described in previous publications (Maurer et al., 2010; Maurer et al., 2007; Bostock et al., 1998). In the following, only a short description of the recording protocol is given. Throughout the entire recording protocol, a stimulus frequency of 1 Hz was chosen. The stimulus strength was adjusted in a feed-back controlled manner for different test paradigms to produce the target response using proportional tracking (Bostock and Rothwell, 1997). First, a stimulus-response curve was recorded by increasing the stimulus current until a maximum sensory nerve action potential (SNAP) was obtained. 40 % of the maximal response was defined as the target response. The current used to reach this target response is referred as “threshold”. Between 40% and 50% of the maximal response size, small changes in stimulus strength evoke major changes in size of the action potential because the slope of the stimulus-response curve is steepest (Maurer et al., 2007). Second, to record the strength duration relationship, stimuli of different widths were applied and the current required to reach the “threshold” was tracked. Third, the recovery of excitability following a single supramaximal stimulus was measured. Therefore, the nerve was stimulated with three different alternating stimulation patterns: (i) control test stimuli tracking the threshold current, (ii) supramaximal conditioning stimuli (140 % of the current needed for a maximal response), (iii) supramaximal conditioning stimuli followed by a test stimulus at variable interstimulus intervals. The effective size of the test action potential after the combined stimulus (iii) was calculated by subtracting (ii) from (iii). Excitability changes were recorded at different conditioning-test intervals between 200 ms and 2 ms in an approximately geometrical sequence. The threshold change was normalized and plotted against the interstimulus interval (fig. 3 A). This method of plotting easily identifies the different phases of the excitability changes of a nerve after a supramaximal stimulus (e.g. relative refractory period, superexcitable period, subexcitable period, s. fig. 3 A) as described by Raymond (Raymond, 1979). Zero on the y-axis represents the control threshold.

Statistical Analysis

All values are given as means ± SD except in figures 2C, 3B and 4C, where SEs were used. To analyse data we used the software QtracP (Version 3/4/2009, ® Institute of Neurology, University College London, UK). Data were tested for a normal distribution with Lillefor’s test for normality. In the preliminary vehicle experiment we used a paired t- test to compare excitability changes between control, intralipid and propofol. A Bonferroni correction was performed to

address multiple comparisons for the same variable. Concentration-response data were depicted as semilogarithmic graphs, with data fitted with regression analysis for the best fit. In the main experiments we used repeated measures ANOVA with Bonferroni corrections to compare the changes of excitability parameters at different propofol concentrations. P-values < 0.05 were considered statistically significant.

Results

Our results showed a reduction of excitability parameters in sensory nerve afferents with increasing concentration of propofol.

The vehicle intralipid alone did not cause any excitability changes

In the first set of experiments with we tested the hypothesis that commercially available propofol affects peripheral nerve excitability and that this effect was caused by the active component propofol. We found no significant effect of intralipid, the vehicle of propofol, on threshold (100.5 ± 8 %, P=0.73), superexcitability (-44 ± 1 %, P=0.57) or peak amplitude response (98 ± 1 %, P=0.52) measurements (Fig. 2). In contrast, a significant change in threshold (131 ± 18 %, P<0.05) and superexcitability (-39 ± 3 %, P<0.05) was immediately observed during the infusion of an equivalent concentration of 500 µMol propofol. Although the peak amplitude decreased (90 ± 7 %) during this infusion, this change was not statistically significant (P=0.06). It is

Excitability changes caused by propofol

An overview of the excitability changes in the second set of experiments are given in Table 1. All the changes in excitability parameters were only partially reversible during the wash-out phase of propofol for reasons that are discussed in the discussion section. The stimulus-response curve shifted towards smaller responses (on the y-axis) with increasing concentrations (Fig. 3 A). Therefore the size of the maximal SNAP (peak response) decreased with increasing concentration (0 µMol: 6.7 ± 2.0 mV; 1000 µMol: 3.7 ± 1.0 mV; P=0.09). The stimulus strength required to reach the peak did not change (no shift on the x-axis). Furthermore, propofol slowed conduction velocity: latency (measured from the start of the stimulus to the peak of the SNAP) increased significantly with increasing concentrations (0

µMol: 0.96 ± 0.07 ms; 1000 µMol 1.10 ± 0.06 ms; P<0.01; Fig. 3). Strength-duration time constant (τSD) steadily increased during the application of higher concentrations of p
opofol (table 1). τSD reversed to the level of the control values after wash-out. However, this effect was statistically not significant. Rheobase remained similar for all measured concentrations including the wash-out (table 1). Much stronger effects on nerve excitability were observed during the recovery cycle (Fig. 4). Propofol prolonged the relative refractory period (RRP) with increasing concentrations (0 µMol: 1.79 ± 1.13 ms; 100 µMol: 2.53 ± 1.38 ms; P<0.01). In parallel, the extent of the superexcitability decreased (0 µMol: -14.0 ± 4.0%; 100 µMol: - 9.5 ± 5.5 %; P<0.01). At the highest concentration of 1000 µMol the nerve did not enter a superexcitable period at all. Therefore, the RRP could not be determined any longer. At this concentration, the current strength desired to reach the target size of the SNAP was higher than the control current during an interstimulus interval of ~ 100 ms. After the wash-out, the nerve was superexcitable again and accordingly, the RRP could be determined again. The maximum change of threshold during the subexcitable period decreased significantly with increasing propofol concentration (0 µMol: 7.5 ± 1.2 %, 1000 µMol: 3.6 ± 1.2 %; P<0.01).

Discussion

The current study showed that propofol modulates axonal nerve excitability of primary sensory afferents in a concentration-dependent way. The threshold tracking technique revealed an involvement of different voltage-gated ion channels.

Slowing of conduction velocity and decrease in peak response imply a block of voltage-gated sodium channels

In our experiments propofol produced a slowing of conduction velocity (calculated as conduction distance divided by latency). Theoretically, three main physiological mechanisms can account for this finding assuming the maintained integrity of the myelin sheath: (i) A decreased number of available voltage-gated sodium channels which are opened during an action potential along the axon. Either, they were in an inactive state or they were blocked by propofol. (ii): Kinetics of voltage gated ion channels was altered. This usually happens with a temperature change (Paintal, 1965). In this study, temperature was maintained stable throughout the whole experiment, therefore we reject this possibility (iii): Repetitive stimulation

of nerve fibres at high frequencies generate an activity-dependent hyperpolarization intracellulary limited by differences in the resting membrane potential prior to activation (Bostock et al., 2003) or a differential effectiveness of the electrogenic sodium pump (Na+/K+-ATPase) (Rang and Ritchie, 1968). In our experiments, the stimulation frequency of 1 Hz was too low to generate activity-dependent hyperpolarization. We therefore the must assume that the most probable explanation for the decrease of conduction velocity is a direct impact of propofol on voltage-gated sodium channels. From previous studies we know that administration of systemic sodium channel blockers slows down conduction velocity at concentrations much lower than needed for conduction block (De Jong and Nace, 1968; Fink and Cairns, 1984; Lang et al., 2007). Furthermore, Aβ-fibers can be blocked at lower concentrations of sodium channel blockers as compared to small-diameter fibres (Fink and Cairns, 1984) and they manifest significantly more slowing of conduction velocity (Fink and Cairns, 1984; Gokin et al., 2001). In line with the interpretation that propofol blocked voltage-gated sodium channels was the observation that the size of the maximal compound action potential decreased with increasing propofol concentration. Again, the most probable explanation is an intrinsic blocking of voltage-gated sodium channels. Blocking of sodium currents lead to a reduced size of peak response (Butterworth and Stricharzt, 1990) by three main mechanisms: (i) Individual fibres within the nerve show a differential slowing pattern leading to a flattening out of the compound action potential; (ii) Reduced availability of fibres by a complete conduction block or, (iii) changes in the amplitude of individual spikes (Fink and Cairns, 1984). Taken together these findings with previously published data produced in other models at similar propofol concentrations (Rehberg and Duch, 1999) we assume that propofol had the capacity to block axonal sodium channels which led to both, conduction velocity slowing and decrease in peak response. We can only speculate about which sodium conductances were blocked by propofol. One possibility is a direct block of transient sodium currents by a local anesthetic-like mechanism within the pore. An equally plausible variant would be that the axons were depolarized in the presence of propofol because persistent Na currents were blocked, leading to a depolarization of the axons and consequently to an inhibition of the number of channels available to deliver transient current.

<u>The recovery cycle reveals involvement of voltage-gated potassium channels</u>

After a supramaximal stimulus a typical sequence of excitability changes of the axonal membrane takes place which is called 'recovery cycle'. After being refractory axons first enter a superexcitable then a subexcitable period before excitability thresholds normalize again (Stys and Ashby, 1990). Underlying mechanisms are complex and involve mainly sodium conductances at short interstimulus intervals (Hodgkin and Huxley, 1952), and capacitive charging of the internode (Barrett and Barrett, 1982), periaxonal accumulation of potassium (Kocsis et al., 1983) and slow potassium conductances at longer interstimulus intervals (e.g. late subexcitability) (Stys and Waxman, 1994; Schwarz et al., 2006).

Virtually all excitability parameters during the recovery cycle showed dose-dependent excitability changes. However, the changes were small and significant effects were only observed at higher concentrations. In general, the method of threshold tracking allows to attribute certain findings to functions of specific voltage-gated ion channels (e.g. sodium or potassium channels, (Burke et al., 2001)). On the other hand, the technique is often not specific enough and the measurements represent a combination of effects on different factors determining excitability. For example, the RRP is most sensitive to changes in persistent sodium conductances (Baker and Bostock, 1997). In our experiments, RRP increased in a dose-dependent manner. But equally or even more affected was the superexcitable period which is also dependent on changes of the resistance of paranodal membrane by voltage-dependent effects on paranodal potassium channels (Burke et al., 2001). At the highest propofol concentration, no superexcitability – and therefore no RRP – was measurable any longer. The induced changes resembled those described in depolarized nerves (Kiernan and Bostock, 2000). Their investigations showed that depolarizing currents induce an abolishment of superexcitability and late subexcitability. This differs from excitability changes caused by hypoxemia, where late subexcitability is not altered (Kiernan and Bostock, 2000). In our experiments, late subexcitability decreased only at higher concentrations of propofol. Since previous studies showed that late subexcitability can be specifically suppressed by blocking slow potassium currents (Schwarz et al., 2006) we may assume that propofol also affects these slow potassium currents.

Limitation of informative value of threshold tracking techniques

Measurements of nerve excitability always reflect a combination of all the factors that determine the excitability of a nervous membrane. Therefore, the overall interpretation of the changes in excitability induced by difference concentration of propofol can not be explained separately to each parameters, but rather as a result together. The main determinant of course is the resting membrane potential of the axonal membrane which is dependent on ion-channels (voltage gated, ligand-gated) ion pumps and temperature (Goldman, 1943) but also on intra- and extracellular proteins, amino acids, pH etc. Whether we eventually measured a direct effect of propofol on voltage-gated ion channels or an indirect effect by affecting factors that alter resting membrane potential and, hence, the function of these channels remains uncertain. A simple and objective means of interpreting these nerve excitability recordings would be by matching them with a computer model (Bostock, 2006). However, to date such modelling only exists in human motor nerve which is difficult to compare with rodent sensory nerve.

Another limitation was the method of drug application in our rodent model. Since the drug does not reach the nerve membrane via vasa nervorum the time constant of the effects cannot be compared to data obtained in human studies and we must assume that all the effects that we measured occurred with a certain time lag. In the preliminary experiments we used a different setup to separate effects of the active component propofol and the vehicle intralipid than for the main experiments. The nerve was (i) desheated and (ii) stimulated with a suction electrode. In this setup smaller stimulation currents were needed and smaller compound action potentials produced. We explained these phenomenons by a shortfall of fibres due to the mechanical impact of the suction electrode and the lacking neural sheet. This was the reason to use nerves with intact neural sheets for the main experiments. With the neural sheet intact 40 minutes wash in time was required to reach a steady state, in comparison to 15 minutes using the desheathed experimental set-up. This suggests that the sheath surrounding the nerve acts to limit the wash-in of propofol to considerable extent. Similarly to the second set of experiments, only a partial washout was observed which also can be explained by the nerve sheath acting as a barrier and explains why the effect of propofol was not fully reversible in our experimental setup.

Implications on pain perception of the excitability changes observed

From clinical practice it is well known that propofol at high concentrations causes pain at the site of injection (Tan and Onsiong, 1998). Mechanisms proposed include the activation of nociceptors by acidic sensing ion channels (Klement and Arndt, 1991) or by an indirect action via the plasma kallikrein-kinin system (Scott et al., 1988). Recent reports suggest a major role for transient receptor potential (TRP) ion channels, especially TRPA1 (Matta et al., 2008;Lee et al., 2008). This effect is only local and occurring at high concentrations which has to be clearly differentiated of systemic effects at much lower concentrations. Propofol has an analgesic effect in subhypnotic concentrations (Hand et al., 2001; Bandschapp et al., 2010) and is able to suppress spinal sensitization (O'Connor and Abram, 1995) and interneuronal activity in cerebral cortex (Woodforth et al., 1999). However, the mechanisms underlying this analgesic effect remain unclear. A possible involvement of the peripheral nervous system has been discussed (Bandschapp et al., 2010) but no investigation so far was designed to look at the periphery. All the electrophysiological (clinical) investigations have so far considered the effect of general anaesthetics on afferent axons as negligible, since axons are not usually considered as neural targets for these agents (Raymond et al., 1991; Sloan and Jäntti, 2008). A recent investigation in pati ents with the same threshold tracking technique used in the present study revealed an effect on voltage-gated sodium channels in Aβ-afferents. But the measurements recorded were not conclusive about whether sodium currents were blocked or the kinetics of the sodium channels were altered by a minimal temperature increase at the stimulation site (Maurer et al., 2010).

In the peripheral nervous system, a well organized interaction of different subtypes of voltage-gated ion channels defines the size, frequency and the speed of an action potential. Even a slight shift in membrane potential of a nerve membrane can lead to severely altered excitability properties, and thereby modulate the information conveyed to the central nervous system (Raymond et al., 1990; Amir et al., 1999). Along this line of argumentation the alteration of the membrane potential in the present study might be important enough to influence pain perception even at a peripheral level and be responsible – at least to some part – for an analgesic effect of low-dose propofol.

Conclusions

This is the first investigation that has systematically studied the effect of propofol on axonal excitability of primary sensory afferents. Our data suggest that the main effect of propofol on myelinated peripheral sensory afferents was an overall decrease in excitability. We could show that propofol reduces nerve excitability in a concentration dependent manner. Based on the existing knowledge of the threshold tracking technique and the interpretation of the resulting data the changes observed could theoretically be explained by a significantly altered function of axonal voltage-gated sodium channels of Aβ-afferents. We therefore also assume that the analgesic effects described in previous studies could be caused at least partially in the peripheral nervous system.

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Table 1 Summary of nerve excitability changes induced by propofol in vitro.

Propofol concentration	0 µMol	10 µMol	100 µMol	1000 µMol	*P*	Wash-out
Latency (ms)	0.96 ± 0.07	1.01 ± 0.09	1.03 ± 0.07	1.10 ± 0.06	**<0.01**	1.07 ± 0.07
Peak (mV)	5.4 ± 2.0	3.7 ± 2.0	3.3 ± 2.4	3.0 ± 2.1	0.09	4.2 ± 2.2
SDTC (ms)	0.12 ± 0.03	0.15 ± 0.08	0.16 ± 0.10	0.17 ± 0.06	0.23	0.12 ± 0.03
Rheobase (mA)	0.34 ± 0.04	0.41 ± 0.10	0.29 ± 0.10	0.37 ± 0.06	0.72	0.38 ± 0.07
Superexcitability (%)	−14 ± 4	− 13 ± 5	− 10 ± 6	n.a.*	**<0.01**	− 8 ± 6
Subexcitability (%)	8 ± 1	8 ± 3	6 ± 3	4 ± 1	**<0.01**	5 ± 2
Relative Refractory Period (ms)	1.79 ± 1.13	1.93 ± 1.25	2.53 ± 1.38	n.a.*	**<0.01**	2.93 ± 1.43
Refractoriness at 2.0 ms (%)	−7 ± 2	−1 ± 2	10 ± 3	45 ± 3	**<0.01**	11 ± 3

All values are given as mean ± SD (n=13). A repeated measures ANOVA was used to compare changes of the measured parameters with increasing concentration of propofol. SDTC = strength-duration time constant. n.a.* = not applicable: At the highest concentration of propofol the nerve membrane did not enter the superexcitable period. Therefore, the relative refractory period and superexcitability were not measurable.

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FIGURE CAPTIONS

Figure 1. Experimental setup.

The saphenous nerve of the rat was placed in synthetic interstial fluid (SIF) which was circulating in an organ bath. The nerve's proximal ending was placed in a separate recording chamber and isolated from SIF by silicone oil. The temperature of the bath was maintained at 32°C and the SIF was constantly gassed with 95% oxygen and 5% carbon dioxide. A second circuit independent from the organ bath contained propofol dissolved in SIF. Within the compartment of the metal ring the nerve was exposed to propofol at different concentrations and also gassed continuously.

Figure 2. Excitability changes caused by vehicle vs. propofol

a. The graphically generated A-fiber compound action potentials (A-CAPs) represent the mean of three A-CAPs of each of the 5 investigated nerves (totally 15 CAPs). Dashed line represents A-CAP waveform during control (left) and washout period 1 (right). Solid line represents A-CAP waveform during 500 µMol intralipid (right) and equally diluted intralipid (left) infusion. No difference in waveform was found after intralipid infusion, but there was a decrease in peak amplitude and a slight slowing of latency (represented by a shift in waveform towards the right) during the infusion of propofol with the vehicle. Arrows indicates the onset of electrical stimulation. **b**. Original recording showing continuous tracking of threshold and superexcitability (measured at an interstimulus interval of 6.3ms) over time in response to 500 µMol propofol and equally diluted intralipid. Note the increase in threshold and decrease in superexcitability during propofol application. During the washout 2, threshold returned only partially towards pre-propofol values. **C**. Average change in peak amplitude, threshold and superexcitability expressed as a percentage of the control values. Data are shown as mean ± SEM. (n = 5).

Figure 3. Peak response and latency changes.

a. The stimulus-response curve shifted downwards on the y-axis with increasing propofol concentration, indicating a smaller response while the stimulus strength remained similar. **b**. Consequently, the peak response after a maximal stimulus decreased. The latency increased (hence, conduction velocity decreased) with increasing concentration of propofol. Error bars are SEM (n=13).

Figure 4. Recovery cycle.

a. After a supramaximal conditioning stimulus excitability changes were recorded at different conditioning-test intervals between 200 ms and 2 ms in an approximately geometrical sequence. The change of currents of the test stimuli to reach the threshold is plotted on the y-axis in a normalized way, whereby zero represents the unconditioned control threshold. Immediately after a supramaximal conditioning stimulus the nerve enters the refractory period. The refractory period ends as soon as the curve crosses the zero line for the first time below zero. Thereafter, the nerve enters a superexcitable period until it becomes subexcitable (second intersection of the curve with the zero line). Normal excitability is restored after 200 ms. The recovery cycle curve shifted to the right with increasing propofol concentrations. At the same time, superexcitability decreased and was completely abolished at the highest propofol concentration. Consequently, the relative refractory period could not be determined (no intersection with zero line). Subexcitability also decreased significantly. All those changes restored only partially after wash-out (dashed line). **b**. Concentration-response curves showed a strong second-order correlation for superexcitability, relative refractory period and subexcitability. Error bars are SEM (n=13). * This parameter could not be determined (s. above).

Conflict of Interest statement

Lisa Neukom: none

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Figure 1

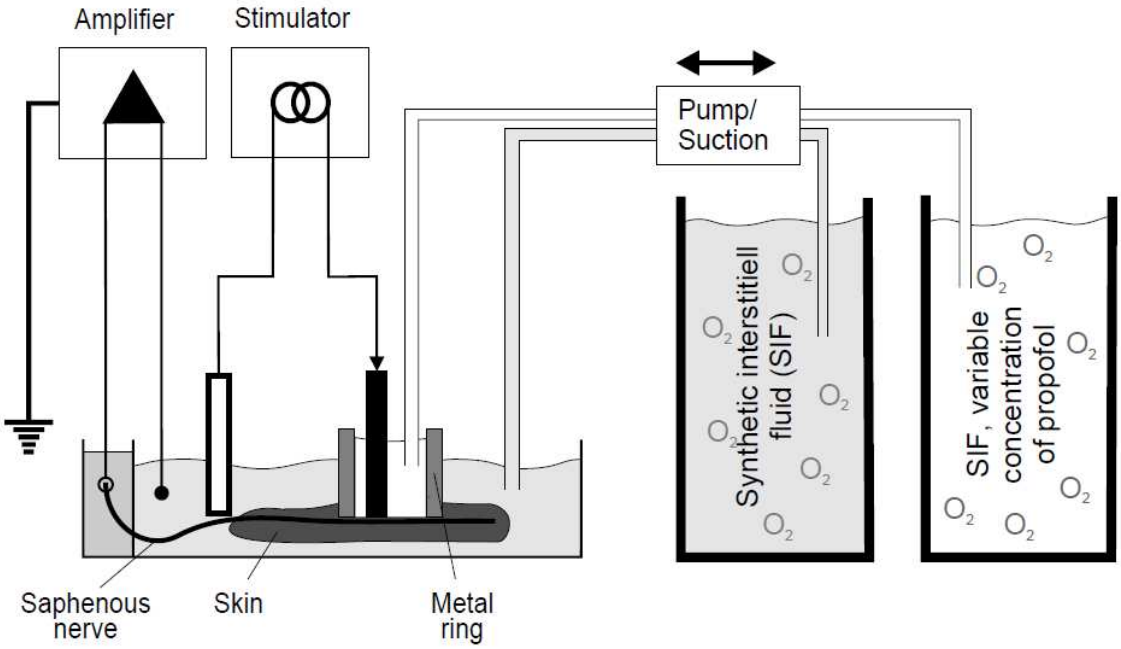


Figure 2

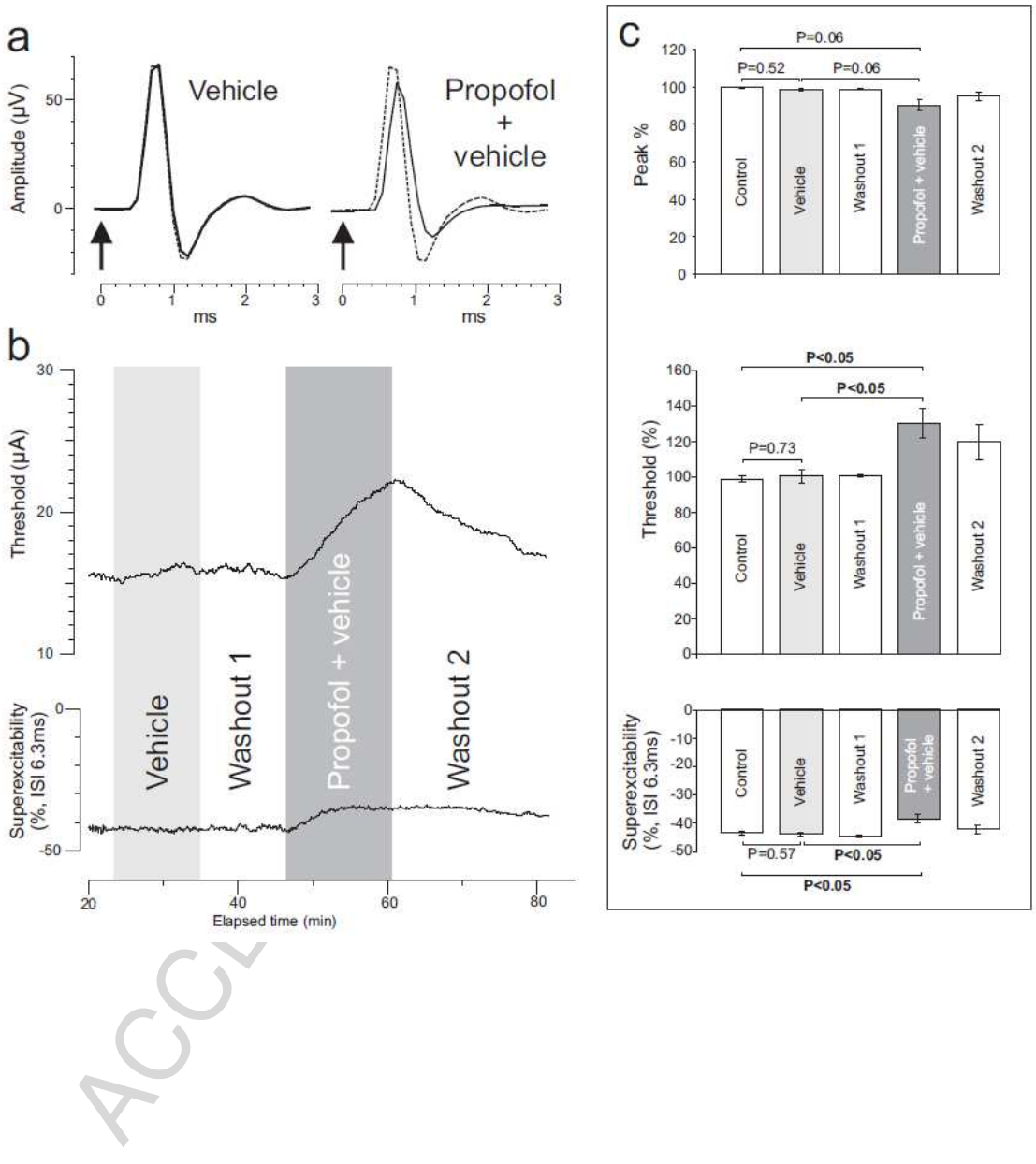


Figure 3

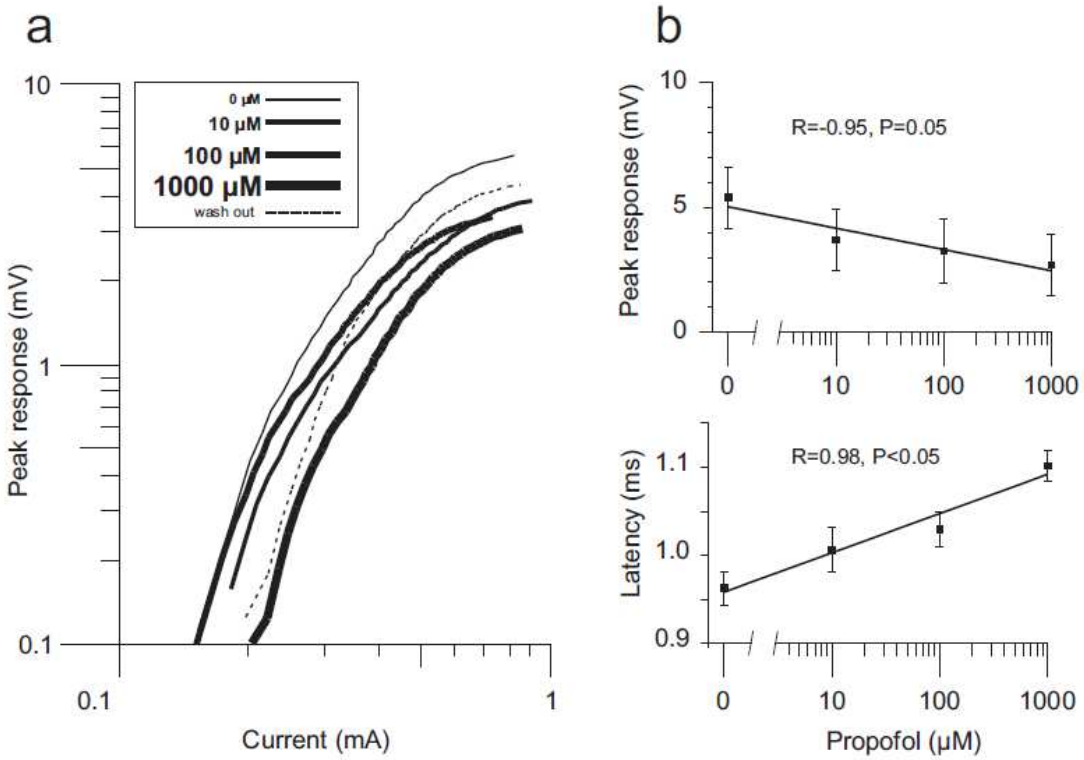
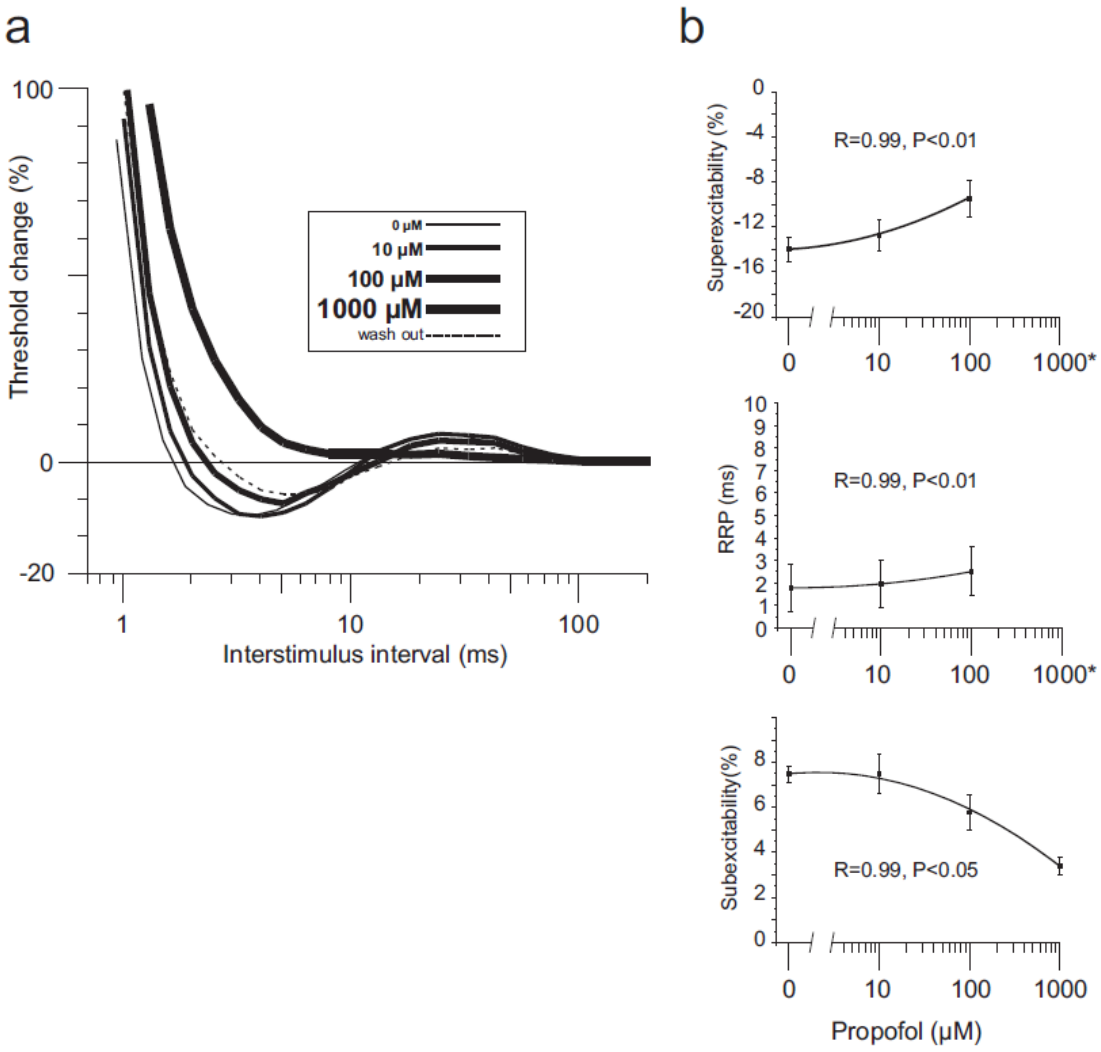


Figure 4



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Conflict of Interest Policy

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Declarations

Life Sciences require that the **corresponding author**, signs on behalf of all authors, a declaration of conflicting interests. If you have nothing to declare in any of these categories then this should be stated.

Conflict of Interest

A conflicting interest exists when professional judgement concerning a primary interest (such as patient's welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). It may arise for the authors when they have financial interest that may influence their interpretation of their results or those of others. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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Signature **Print name**

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